REMARKS

With entry of the instant amendment, claims 1, 2, 4, 6, 8, 10, 12, 34 and 37-39 have been amended and claims 20-27 and 31-33 have been newly cancelled. Claims 1-16, 34-39, 41, and 42 are therefore pending in the application. Applicants specifically reserve the right to pursue the cancelled claims or subject matter in one or more subsequent applications.

The amendments to the claims add no new matter.

For convenience, the objections/rejections will be addressed in the order set forth in the Office Action mailed January 29, 2004.

Applicants thank the Examiner for the interview on April 20, 2004 in which the enablement and written description rejections were discussed. In particular, the rejections in regard to pyroglutamic acid and activity of the claimed sequences that comprise a glutamine at the N-terminus were discussed.

For convenience, the objections/rejections are presented in the order set out in the Office Action mailed January 29, 2004.

Objection to Claim 6

This rejection is moot in view of the amendments to the claims.

Rejections under 35 U.S.C. § 112, first paragraph--enablement

Claims 1-16 remain rejected as allegedly not enabled. In particular the Examiner contends that there is unpredictability in terms of the activity of ONCONASE®-related ribonucleases with respect to the residue at position 1. She argues that the prior art teaches that the N-terminal pyroglutamic acid is important for activity and that it would require undue experimentation to determine whether ribonucleases with residues other than pyroglutamic acid are active. Applicants respectfully traverse.

First, the Examiner cites page 44, lines 20-23 as allegedly teaching that the N-terminal pyroglutamic acid is critical for activity. However, this passage explicitly refers to recombinant ONCONASE®. It notes that recombinant ONCONASE® was not very cytotoxic in

the experiments shown in table II, page 45 of the specification. At lines 23-27, the specification also teaches that recombinant *Rana pipiens* liver RNases were more active than recombinant ONCONASE® and moreover, that such RNases do not display the same dependence as ONCONASE® on the N-terminal pyroglutamic acid residue for correct hydrogen bonding. The data in Table II in fact demonstrate this: not only does recombinant rapLR1 have cytotoxic activity, but rapLR1 with a serine substituted for the glutamine at position 1 also exhibits cytotoxic activity.

In order to expedite prosecution, Applicants are obtaining a Declaration under 37 C.F.R. § 1.132 that provides additional evidence that the claimed recombinant rapLR1 sequences that have a glutamine at the N-terminus have RNase activity in a direct RNase activity as well as in a cytotoxicity assay. The Declaration will also include the results of a protein sequence determination that shows that the glutamine at position 1, it is not blocked, *i.e.*, that it is not pyroglutamic acid. Accordingly, the Declaration provides additional evidence that rapLR1 having a glutamine at position 1 is active.

With regard to the art cited on page 4 of the specification, the Examiner also argues that there is a discrepancy between the disclosure in the specification at Table 1 and lines 20-23 of page 44, and the reference Newton *et al.* (*Protein Engineering* 10:463-70, 1997). Table 1 indicates that the recombinant ONCONASE® is one in which serine places the glutamine at position 1. It's not clear why the Examiner believes that the cited art is of particular relevance to Table 1. As noted above, the specification teaches that rapLR1 sequences do not exhibit the same degree of dependence on the N-terminal residue as ONCONASE. Further, the state of this art in terms of knowledge of structure function relationships of RNase A superfamily members is advanced, as evidenced by the extensive literature in this field. *See, e.g.*, the references cited at page 16 in the specification, as well as references cited by the Examiner (*e.g.*, Figure 5 of Newton *et al.*, *Protein Engineering, supra*, provides structural comparisons). Accordingly, the teachings of the specification in conjunction with the knowledge in the art enables one of skill to identify or design RNases molecules as set forth in the claims.

Next, she alleges that the art teaches that ribonucleases with methionine at position 1 do not have activity, citing Newton et al. *Biochemistry* 37, 5173-5183, 1998. For

example, the Examiner cites the first line of the abstract. However, the first line of the abstract does not relate to methionine at position 1. This aspect of the rejection is additionally confusing because the Examiner then points to the same references as teaching that Met(-1) constructs do in fact have activity.

Last, the Examiner cites Chen *et al.* (*Nucleic Acids Res* 28:2375-82, 2000) as teaching that SEQ ID NO:2 is merely a truncated putative ribonuclease. However, the Examiner provides no evidence or reasoning as to why Chen *et al.* would lead one of skill to believe that the claimed sequences are not active ribonuclease molecules. The specification teaches recombinant rapLR1 has activity, *e.g.*, Table II. Example 3 also teaches how the cDNA sequence was obtained. Clone 5a1b cDNA encodes a sequence characteristic of a signal peptide that is followed by the highly conserved RNase sequence (page 39, line 30 bridging to page 40, line 3). The specification teaches that an RNase of the invention may optionally have a signal peptide (*see, e.g.*, page 17, lines 3-10; page 18, lines 16-19). However, no argument is advanced by the Examiner based on Chen as to why one of skill would believe that inclusion of the additional signal sequence would be required to enable the scope of the invention.

In view of the foregoing, Applicants submit that the claims are enabled for the full scope. Applicants therefore respectfully request withdrawal of the rejection.

Specification

The Examiner required correction of "Onconase" in the specification to "ONCONASE". The specification has been amended accordingly.

The Examinerr also objects to the specification at page 2, line 10 for stating that ONCONASE® has an N-terminal glutamine. She maintains this is incorrect because Ardelt, *et al.* (*J. Biol. Chem* 266:245-251, 1991) teaches that the N-terminal residue is glutamic acid. Applicants disgree. Ardelt teaches that the N-terminal residue is pyroglutamic acid. Applicants note that the passage indicted by the Examiner refers to ONCONASE expressed in bacterial. Pyroglutamic acid is not in and of itself encoded by an expression vector. Attached as Appendix B is a protein database listing for Onconase, which shows glutamine as the N-terminal residue and a database listing for a *Rana pipiens* onconase precursor, which shows that the nucleic acid

encodes a glutamine at the N-terminal position of the RNase portion of the sequence. Thus, the sentence at issue is correct. Applicants therefore respectfully request withdrawal of the objection.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-6 were rejected as allegedly indefinite. The Examiner maintains that claim 1 is not clear in the recitation of "substantial identity". Further, the rejection alleges that the claims are not clear in the use of "amino terminal end" and "position 1". In order to expedite prosecution, the claims have been amended. Applicants therefore respectfully request withdrawal of the rejection.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-16 were rejected as allegedly lacking adequate written description. The Examiner maintains that the claims are not supported by sufficient description of the characteristics of the genus of claimed proteins. In particular, the Examiner argues that only a partial structure of a sequence is provided, and that in view of Chen *et al.*, *supra*, Applicants did not have possession of the full length protein sequence comprising SEQ ID NO:2. Applicants respectfully traverse.

With regard to the allegation that Applicants do not disclose a "full-length" protein sequence comprising SEQ ID NO:2, Applicants note that the sequence of the *Rana pipiens* Clone 5a1b cDNA and the translated amino acid sequence with signal peptide are disclosed in SEQ ID NOs:27 and 28 of the application. This would appear to be what the Examiner considers as a "full-length" protein based on the citation of Chen *et al.* in the argument.

Applicants further submit that the instant specification meets the requirements for providing proper written description support. The claims recite a reference sequence as well as specifying particular residues that characterize the claimed genus. Further, the claims recite a functional feature. These elements are fully supported in the specification. For example, the specification teaches that many sequences that are related to the claimed sequences are known

(*see, e.g.,* the references cited at page 16 in the specification) and have been evaluated. Thus, this is a highly advanced field in terms of knowledge of structure/function relationships.

In addition, the specification also provides description of particular residues that are maintained in the overall structure and guidance and examples of modifications to the structure (*see, e.g.,* the extensive description beginning on page 16 of the specification) that can be made. Moreover, the specification teaches how to evaluate the activity of the sequences of the invention (*e.g.,* Example 7) and provides data showing that exemplary sequences are active (*see, e.g,,* Table II). The Federal Circuit has held that "[a] description of a genus of [DNAs] may be achieved by means of a recitation of ... structural features common in the members of the genus." *The Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1569 (Fed. Cir. 1997). The claimed genus is characterize by both structural and functional features recited in the claims and described in the specification. Accordingly, the claims are fully supported by proper written description support.

Applicants therefore respectfully request withdrawal of the rejection.

Rejections under 35 U.S.C. § 112, first paragraph--enablement

Claims 5 and 11 were rejected as allegedly not enabled. The basis of the rejection appears to be the Examiner's contention that glutamine cannot be cyclized to pyroglutamic acid. First, as shown in Appendix B, the nucleic acid that encodes onconase precursor in fact encodes a glutamine at the N-terminus. Contrary to the Examiner's assertions, it is well known that glutamine can cyclize to pyroglutamic acid. For example, a simple internet search identified numerous references discussing cyclization of glutamine to pyroglutamic acid. An exemplary reference is attached as Appendix C. The reference was obtained from the following site: brc.se.fju.edu.tw/plans/slides/030516/properties.pdf. The file provides the reference source on page 1. Pages 7 and 8 disclose that at the N-terminus of a protein, spontaneous or enzyme-catalyzed cyclization, with the elimination of ammonia, can take place, forming pyroglutamyl residues. A structure showing the cyclization of N-terminal glutamine to pyroglutamic acid is shown on page 8. In view of the foregoing, Applicants respectfully request withdrawal of the rejection.

Claims 34-39 were also rejectd as allegedly not enabled. The basis of the rejection appears to be the argument that the specification does not teach that the proteins as claimed, having glutamine at position 1, exhibit cytotoxicity. Applicants respectfully traverse.

Table II of the specification presents data showing that recombinant RNase sequences having glutamine at the N-terminus are cytotoxic to a variety of cell lines. The data in Table II further show that recombinant molecules in which serine is substitute for glutamine are also cytotoxic. The assertion that Applicants do not show activity of the claimed proteins therefore is incorrect. Applicants therefore respectfully request withdrawal of the rejection.

Double patenting

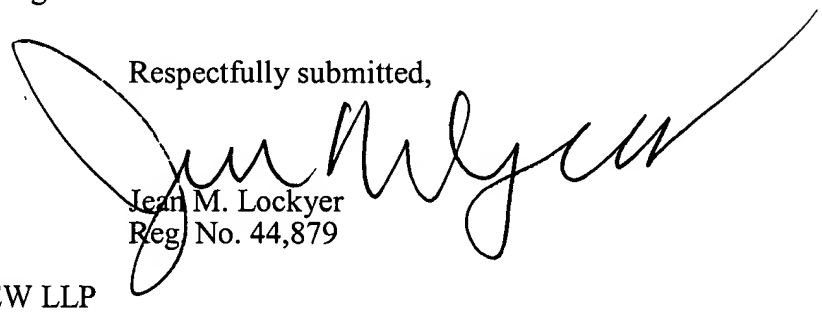
The rejection alleges that claims 34 and 37 are substantially duplicate claims. Applicants submit that the scope differs. Claim 34 recites a ribonuclease expressed from recombinant DNA; claim 37 recites a cytotoxic reagent comprising a sequence selected from the same Markush group. There is no recitation in claim 37 that the ribonuclease is expressed from recombinant DNA. Therefore the claims are not substantially duplicated. Applicants therefore request withdrawal of the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



Jean M. Lockyer
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Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
JML:jml
60267219 v1

Table II Cytotoxicity in human tumor cells of recombinant RNAses (IC_{50}(nM))

Cell line	Tumor type	rec ONCONASE®	rec rapLR1	**Fold Increase**	rec rapLR Q1S	**Fold Increase**	rec RACOR	**Fold Increase**
SF539	Glioma	29,200	2,300	**13**	417	**70**	1,300	**22**
HS578T	Breast	>8,300	8,300	**>1**	670	**>8**	2.500	**>3**
ACHN	Kidney	26,700	3,300	**8**	1,580	**17**	1,000	**11**
Malme	Melanoma	25,000	580	**43**	750	**33**	1,000	**25**
MCF7	Breast	>8,300					320	**26**

Annotated Marked-up Specification Page

Table II Cytotoxicity in human tumor cells of recombinant RNAses (IC_{50} (nM))

Cell Line	Tumor type	rec ~~Onconase~~ ONCONASE®	rec RaPLR1	Fold Increase	rec RAPLR Q1S	Fold Increase	rec RACOR	Fold Increase
SF539	Glioma	29,200	2,300	13	417	70	1,300	22
HS578T	Breast	>8,300	8,300	>1	670	>8	2,500	>3
ACHN	Kidney	26,700	3,300	8	1,580	17	1,000	11
Malme	Melanoma	25,000	580	43	750	33	1,000	25
MCF7	Breast	>8,300					320	26

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            created: Aug 1, 1991.
            sequence updated: Feb 1, 1994.
            annotation updated: Jun 15, 2004.
            xrefs: gi: 494422
            xrefs (non-sequence databases): InterProIPR001427, PfamPF00074,
            ProDomPD000535, SMARTSM00092, PROSITEPS00127
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  ORGANISM  Rana pipiens
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REFERENCE   1  (residues 1 to 104)
  AUTHORS   Ardelt,W., Mikulski,S.M. and Shogen,K.
  TITLE     Amino acid sequence of an anti-tumor protein from Rana pipiens
            oocytes and early embryos. Homology to pancreatic ribonucleases
  JOURNAL   J. Biol. Chem. 266 (1), 245-251 (1991)
  MEDLINE   91093131
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            TISSUE=Embryo
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  TITLE     Refined 1.7 A X-ray crystallographic structure of P-30 protein, an
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  JOURNAL   J. Mol. Biol. 236 (4), 1141-1153 (1994)
  MEDLINE   94166079
   PUBMED   8120892
  REMARK    X-RAY CRYSTALLOGRAPHY (1.7 ANGSTROMS).
COMMENT     On Mar 25, 1994 this sequence version replaced gi:133145.
            [FUNCTION] Basic protein with antiproliferative/cytotoxic activity
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            vivo. It exhibits a ribonuclease-like activity against high
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  AUTHORS   Liao,Y.D., Wang,S.C., Leu,Y.J., Wang,C.F., Chang,S.T., Hong,Y.T.,
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  TITLE     The structural integrity exerted by N-terminal pyroglutamate is
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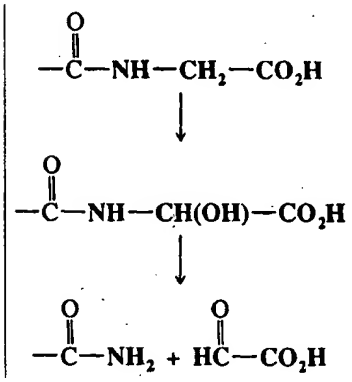
PROPERTIES AND REACTIONS OF AMINO ACIDS

major source: Proteins, G. Allen Ed. Vol.1(1997), JAI Press INC, pp 9-18

Glycine (甘胺酸)

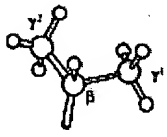
The **simplest amino acid**, unique among the protein amino acids in its **lack of dissymmetry, glycine frequently plays an important role in protein structures where the lack of β-carbon atom permits a substantially greater degree of conformational flexibility and attainable conformational space than for any other residue**. Glycine is thus **often located in tight turns**, and in positions where bulky side chains would sterically prevent close packing of helices (as in collagen) or binding of substrates. The absence of a sterically hindering side chain also **confers greater than normal chemical reactivity at adjacent peptide bonds. For example, Asn-Gly sequences can form cyclic imide structures, with deamidation, much more readily than other Asn-Xaa sequences**. Glycine also contributes to sites recognized by enzymes catalyzing specific modifications of proteins, such as the signal sequences for **N-terminal myristoylation and arginine methylation. The nitrogen atom in C-terminally amidated peptide hormones is contributed by a glycine residue in the precursor peptides.**



Alanine (丙胺酸)

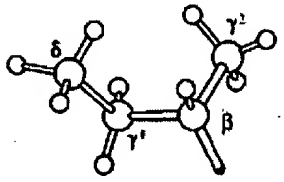
One of the **most abundant amino acid residues** in proteins, alanine is **weakly hydrophobic.** It is often chosen as a **substitute for other amino acid residues in specific mutagenesis experiments designed to test the functional importance of particular side chains**. Perhaps surprisingly, it has been found that a large number of residues may be changed to alanine without significant alteration of the tertiary structures of some proteins. As is the case with the other aliphatic residues, valine, isoleucine, and leucine, chemical reactivity is very low, and this is reflected in the lack of covalent modifications of these residues in proteins.

Valine (纈胺酸)

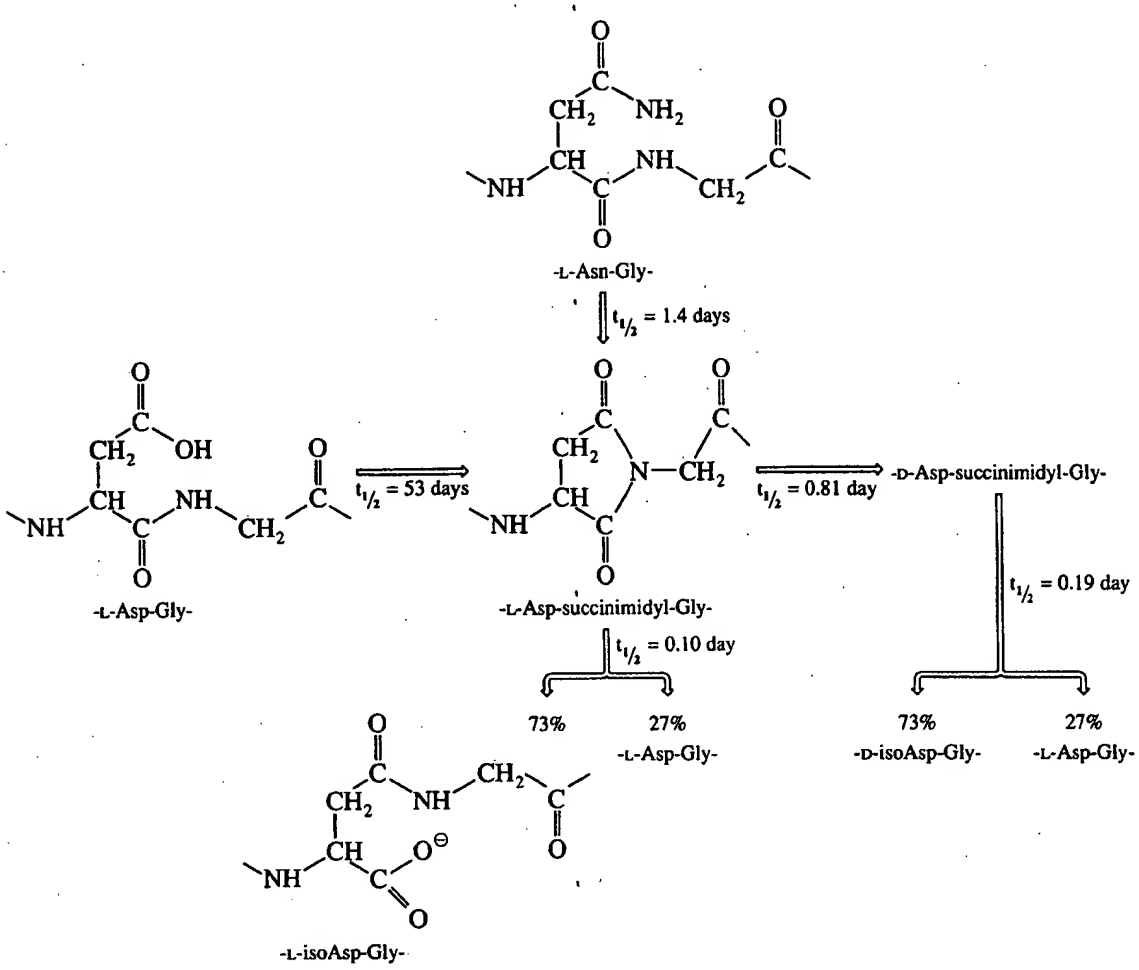


The methyl substituent on the β-carbon of this moderately hydrophobic residue reduces conformational flexibility and confers steric hindrance to chemical reactions at the adjacent peptide bonds, particularly where the adjacent residue also bears a **β-branched side chain (valine or isoleucine)**.

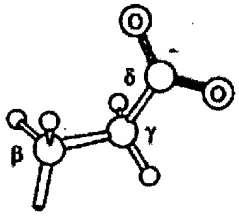
Isoleucine (異白胺酸)



As with valine, the **β-branched side chain** sterically hinders reactions at adjacent peptide bonds. The hydrophobic side chain prefers a location within the interior of folded protein structures, and the β-sheet secondary structure also accommodates the side chain more readily than does an α-helix. Isoleucine has a second asymmetric center. Inversion of the configuration at the β-carbon (which can occur during vigorous acid treatment with HI) yields L-alloisoleucine, a diastereoisomer with different chemical and physical properties.

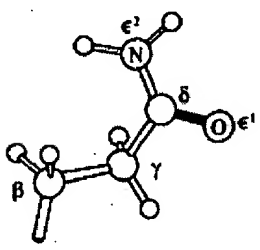


Glutamic acid (麩胺酸)



The **carboxyl group** of glutamic acid residues has a higher intrinsic pKa (around 4.5) than that of aspartic acid residues, but in general has similar functions and properties in contributing to electrostatic and hydrogen bonding interactions within proteins and with ligands, including metal ions. Glutamic acid residues do not, however, possess unusual peptide bond lability, nor are they essential active-site residues in acid proteases. *In vivo* modificaton to γ-carboxyglutamic acid residues is essential to the function of various blood-clotting proteins, such as prothrombin, where the malonic acid group enhances affinity for Ca^{2+}.

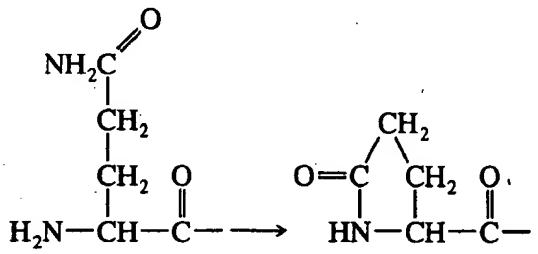
Glutamine (麩醯胺酸)



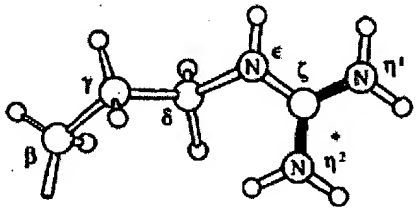
The properties of glutamine are in general similar to those of asparagine, although, as the amide of an acid weaker than aspartic acid, glutamine residues are less labile than asparagine residues. However, at the N-terminus of a protein, spontaneous or enzyme-catalyzed cyclization, with elimination of ammonia, forming

pyroglutamyl residues, takes place. Some glutamine side chains are substrates for transglutaminases, which catalyze linkage to lysyl side chains.

- **Cyclization of N-terminal Gln to form pyroglutamic acid**



Arginine (精胺酸)



The strongly basic (pKa around 12.0) **guanidine group** of arginine residues is protonated at all physiologically relevant pH values. The positive charge, together with multiple hydrogen bond donating capacity and a high degree of polarity, ensures surface localization of arginine side chains, except in rare instances of formation of buried guanidinium-carboxylate salt bridges. Arginine residues function in proteins generally as positively charged groups, contributing to the binding of negatively charged ligands such as phosphate and phosphate esters, including nucleic acids. Together with lysine residues, arginine residues provide positively charged signals for membrane protein assembly, pro-protein cleavage, and nuclear and nucleolar localization. Rather unreactive chemically at neutral pH, arginine residues yield cyclic, frequently fluorescent, products with α- or β-diketones and related compounds at alkaline pH. 1n vivo, methylation and ADP-ribosylation are important modifications.

It consists of three nonpolar methylene groups and the strongly basic δ-guanido group. With a pKa value usually of about 12, the guanido group is ionized over the entire pH range in which proteins exist naturally.

The ionized guanido group is planar as a result of resonance. and the positive charge is effectively distributed over the entire group. In the protonated form, the guanido group is unreaetive, and only very small fractions of the nonionized form are present at physiological pH values